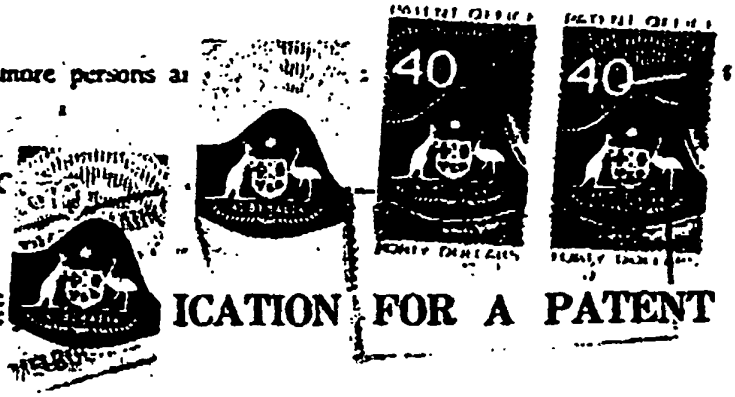
(CONVENTION. By one or more persons as



CONVENTIONAL APPLICATION FOR A PATENT

(1) State full name of Applicant or Applicants, followed by address (es).

XXX We HOECHST AKTIENGESELLSCHAFT,

of 45 Bruningstrasse, D-6230 Frankfurt/Main 80,

Federal Republic of Germany.

(2) State title of invention

hereby apply for the grant of a Patent for an invention entitled:(2)

IMPROVED UTILIZATION OF PLANT-UTILIZABLE NITROGEN BY CROP PLANTS WITH OVEREXPRESSION OF GLUTAMINE SYNTHETASE.

(3) If any, insert number (s) of basic application (s)

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered (3)

P 37 19 053.9

(4) State name of State, Country or Countries, and basic date or dates

for a patent or similar protection made in (4) Federal Republic of Germany on 6th June, 1987.

My/Our address for service is Messrs. Edwd. Waters & Sons, Patent Attorneys, 50 Queen Street, Melbourne, Victoria, Australia.

DATED this 2nd day of June, 1988

(5) Signature of Applicant or Agent

HOECHST AKTIENGESELLSCHAFT

By: D.B. MISCHLEWSKI

Form 7

COMMONWEALTH OF AUSTRALIA

Patents Act 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION UNDER PART XVI. FOR A PATENT.

In support of the Convention application made under Part XVI. of the Patents Act 1952 by HOECHST AKTIENGESELLSCHAFT of 45, Brüningstrasse, D-6230 Frankfurt/Main 80, Federal Republic of Germany for a patent for an invention entitled:
IMPROVED UTILIZATION OF PLANT-UTILIZABLE NITROGEN BY CROP PLANTS WITH OVEREXPRESSION OF GLUTAMINE SYNTHETASE

We, Bernhard Beck, 4 Drosselweg, D-6246 Glashütten/Taunus,
Franz Lapine, 2 Sandweg, D-6244 Kelkheim (Taunus),
Federal Republic of Germany
do solemnly and sincerely declare as follows:

1. We are authorized by HOECHST AKTIENGESELLSCHAFT the applicant for the patent to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was made in the Federal Republic of Germany
under No. P 37 19 053.9 **on** June 6, 1987
by HOECHST AKTIENGESELLSCHAFT

3. a) Peter Eckes, 18 Am Flachsland, D-6244 Kelkheim (Taunus)
b) Günter Donn, 35 Sachsenring, D-6248 Hofheim am Taunus
c) Arno Schulz, 22 Stauffenstraße, D-6234 Hattersheim am Main
d) Friedrich Wengenmayer, 38 Am Geyenbach, D-6248 Hofheim am Taunus
a) - d) Federal Republic of Germany

I/we/are the actual inventor(s) of the invention and the facts upon which HOECHST AKTIENGESELLSCHAFT

is entitled to make the application are as follows:

The said HOECHST AKTIENGESELLSCHAFT

is the assignee of the said
Peter Eckes, Günter Donn, Arno Schulz, Friedrich Wengenmayer

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Frankfurt/Main, Federal Republic of Germany

this 17th day of May 1988

To the Commissioner of Patents

HOECHST AKTIENGESELLSCHAFT

Prokurist Authorized Signatory

ppa. Beck i.V. Lapine

PAT 510

(12) PATENT ABSTRACT (11) Document No. AU-A-17321/88
(19) AUSTRALIAN PATENT OFFICE

(54) Title
IMPROVED UTILIZATION OF PLANT-UTILIZABLE NITROGEN BY CROP PLANTS WITH OVEREXPRESSION OF GLUTAMINE SYNTHETASE

(51)4 International Patent Classification
A01H 001/00 A01H 005/00 C07G 017/00 C12N 015/00
C12N 005/00 A01G 001/00 A01G 007/00

(21) Application No. : 17321/88 (22) Application Date : 03.06.88

(30) Priority Data

(31) Number (32) Date (33) Country
3719053 06.06.87 DE FEDERAL REPUBLIC OF GERMANY

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(57) Claim

Improved utilization of plant-utilizable nitrogen by crop plants with overexpression of glutamine synthetase

Glutamine synthetase is a plant enzyme which occupies a central position in the assimilation of ammonia and in regulating the nitrogen metabolism. The Patent Application WO 86/02097 (PCT) describes the construction of plant cells or plants which have the ability of synthesizing glutamine synthetase in a significantly greater amount than so-called wild-type plants. Plant cells or plants of this type are resistant to glutamine synthetase inhibitors, for example to the herbicide phosphinothricin.

It has now been found, surprisingly, that selected plants which have undergone mutation or modification by genetic manipulation and which produce glutamine synthetase in greater amounts than do wild-type plants utilize more efficiently the nitrogen source necessary for growth.

1. A method for improving the nitrogen utilization in crop plants, which comprises bringing about glutamine synthetas verproduction in the these plants.

2. A method for cultivating crop plants under conditions which are low in plant-utilizable nitrogen, which comprises cultivating crop plants which overproduce glutamine synthetase.

4. A method as claimed in one or more of claims 1 to 3, wherein the glutamine synthetase overproduction is brought about by selection, mutation or genetic manipulation of the plants or plant cell.

11. A plasmid containing the glutamine synthetase gene from Medicago sativa and the promoter to one of the following genes:

a) Gene 1' or gene 2' of the TR-DNA from Agrobacterium tumefaciens A6,

b) ST-LS1 from Solanum tuberosum or

c) 35S transcript from cauliflower mosaic virus.

Form 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-69

COMPLETE SPECIFICATION

(ORIGINAL)

Class Int. Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority :

Related Art :

Name of Applicant : HOECHST AKTIENGESELLSCHAFT

Address of Applicant : 45 Brunlngstrasse, D-6230 Frankfurt/Main 80, Federal Republic of Germany.

Actual Inventor: PETER ECKES, GUNTER DONN, ARNO SCHULZ and FRIEDRICH WENGENMAYER.

Address for Service : EDWD. WATERS & SONS, 50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

IMPROVED UTILIZATION OF PLANT-UTILIZABLE NITROGEN BY CROP PLANTS WITH OVEREXPRESSION OF GLUTAMINE SYNTHETASE

The following statement is a full description of this invention, including the best method of performing it known to us

HOECHST AKTIENGESELLSCHAFT Dr.KL/gm HOE 87/F 166

Description

Improved utilization of plant-utilizable nitrogen by crop plants with overexpression of glutamine synthetase

Glutamine synthetase is a plant enzyme which occupies a central position in the assimilation of ammonia and in regulating the nitrogen metabolism. The Patent Application WO 86/02097 (PCT) describes the construction of plant cells or plants which have the ability of synthesizing glutamine synthetase in a significantly greater amount than so-called wild-type plants. Plant cells or plants of this type are resistant to glutamine synthetase inhibitors, for example to the herbicide phosphinothricin.

It has now been found, surprisingly, that selected plants which have undergone mutation or modification by genetic manipulation and which produce glutamine synthetase in greater amounts than do wild-type plants utilize more efficiently the nitrogen source necessary for growth.

Hence the invention relates to:

a method for improving the nitrogen utilization in crop plants, which comprises bringing about glutamine synthetase overproduction in these plants,

a method for cultivating crop plants under conditions which are low in plant-utilizable nitrogen, which comprises cultivating crop plants which overproduce glutamine synthetase,

a method for generating crop plants, which comprises bringing about glutamine synthetase overproduction in these plants, and providing them with an amount of utilizable nitrogen which without glutamine synthetase overproduction did not result in utilizable growth, and

the use f glutamine synth tase overproduction for improving th utilization of plant-utilizable nitrog n in cr p plants.

The invention also relates to special plasmids which contain a gene structure resulting in glutamine synthetase overproduction, and to crop plants which overproduce glutamine synthetase by reason of a gene structure of this kind.

Figure 1 describes the conversion of the natural glutamine synthetase gene from Medicago sativa, in each case cloned into the commercially available vector pUC12, into a form shortened by the 5'-untranslated region. The gene region is depicted in each case as a thick line, and the polylinker region of the vector is depicted as a thin line. The restriction enzymes (all of which occur in the pUC12 polylinker) are abbreviated in part, for example HindIII as "HIII", EcoRI as "Eco", BamHI as "Bam", and in the case of the enzymes AvaI, PstI, SacI, SalI, SmaI and XbaI the "I" has not been reproduced.

Preferred embodiments of the invention are described in detail hereinafter.

In principle, the invention can be carried out with all crop plants which overproduce glutamine synthetase. This group of plants includes all the selected, mutated or genetically manipulated crop plants whose cells produce significantly greater amounts of glutamine synthetase than does the corresponding wild-type. Suitable crop plants belong both to the monocotyledons and to the dicotyledons. Among the monocotyledons, cereal plants, especially corn, wheat and rice, are preferably used. The dicotyledons used are fodder plants such as alfalfa or clover; tobacco, pepper, groundnuts, but especially vegetable species such as potatoes, t matoes; brassica species such as cabbage or rape; l gum s such as peas or b ans (Phaseolus, Vicia or Vigna), ro ts such as sugar-

beet, beetroot or carrot, as well as soybeans.

As already mentioned, the Patent Application WO 86/02097 describes the construction of plant cells or plants which are able to produce glutamine synthetase in greater amounts. Plants prepared in this way or regenerated from cultures of plant cells in this way can be used according to the invention. Suitable plants are both those in which the glutamine synthetase gene which occurs naturally in the plant or plant cell is formed, owing to appropriate selection pressure, in a higher copy number, and those plants which have been mutated with conventional agents or have undergone modification by genetic manipulation and which can be prepared using various methods. For example, it is possible to construct a plant cell with the following gene combination:

a) a genetic sequence which codes for glutamine synthetase and is expressed in the plant cell can be linked to

b) a second genetic sequence which is able to increase the expression of the genetic sequence mentioned under a), i.e. to increase significantly the amount of active glutamine synthetase in the cell.

A significant increase in the amount of active glutamine synthetase is defined as the increase which suffices for the particular plant to be able to grow even under conditions which, in respect of the amount of plant-utilizable nitrogen, for the wild-type result in growth which is diminished and useless for practical purposes.

A gene combination of this type can be inserted into the genome of the plant cell directly or with the aid of a vector, with the Ti plasmid of Agrobacterium tumefaciens being preferred here. In this connection, it is possible for the said glutamine synthetase gene sequence to be both homologous and heterologous for the plant or the cell culture. In the case of a heterologous gene

sequence, this can originate both from other organisms, for example microorganisms or animals, as well as, preferably, from other plant species. Examples of preferred plants from which the glutamine synthetase gene can be obtained are Arabidopsis thaliana, Phaseolus vulgaris, Medicago sativa, Nicotiana plumbagenifolia, Pisum sativum or Nicotiana tabacum.

The second gene sequence can be a promoter sequence, namely the sequence of a promoter with high expression in plants. A promoter of this type needs to meet only one important condition, namely that, in combination with the glutamine synthetase gene, it expresses the enzyme in the plant cells in significantly greater amounts than is the case in the unmodified wild-type. The promoter can be both homologous and heterologous for the host cell. In the case of heterologous promoters it is preferable to use gene sequences from other plant species as well as from microorganisms which live in symbiosis with plants, or from plant viruses.

Suitable examples are the promoter of the small subunit (ss) of ribulose-biphosphate carboxylase, as well as the promoter of the protein which binds chlorophyll a/b [WO 86/02097; A. Cashmore, Genetic Engineering of Plants, An Agricultural Perspective, Plenum, New York 1983, pages 29-38; Coruzzi G. et al., The Journal of Biological Chemistry 258, 1399 (1983); Dunsmuir, P., et al., Journal of Molecular and Applied Genetics 2, 285 (1983)].

However, the promoters of the following genes are preferably used:

a) Gene 1' of the TR-DNA from Agrobacterium tumefaciens A6 (Velten et al., EMBO J. 3, 2723 (1984))

b) Gene 2' of the TR-DNA from Agrobacterium tumefaciens A6 (Velten et al., loc. cit.)

c) ST-LS1, a leaf- or stem-specific gene whose regulation is light-dependent, from Solanum tuberosum (Eckes et al., Mol. Gen. Genet. 205, 15 (1986))

d) 35S transcript from cauliflower mosaic virus (Pietrzak et al., Nucl. Acids Res. 14, 5857 (1986)).

The gene constructions obtained with these promoters are new, and the invention likewise relates to them.

It is particularly preferable to couple to these promoters the gene of glutamine synthetase from Medicago sativa which is described by Tischer et al. (Mol. Gen. Genet. 203, 221 (1986)) (and proposed in EP-A 0,239,801) after it has been appropriately modified by various methods of genetic manipulation, such as restriction cleavages, ligations etc. (Maniatis et al., Molecular Cloning, Cold Spring Harbor, 1982). However, it is also possible to use glutamine synthetase genes from other plant species together with these promoters. It is furthermore possible to use mutated genes as proposed in EP-A 0,240,792.

Promoter-glutamine synthetase gene constructions of this type can be introduced by methods known per se both into appropriate plant cell cultures or protoplasts, from which plants can then be regenerated in each case, as well as into the complete crop plant [Potrykus et al., Plant Mol. Biol. Reporter 3, 117 (1985); Horsch et al., Science 227, 1229 (1985)].

Plants with increased glutamine synthetase production can likewise be obtained by mutation of the wild-type plants. The mutation can be brought about both with chemicals, such as, for example, ethyl methanesulfonate or nitrosoguanidine, and by irradiation, such as, for example, with X-rays or UV rays. Mutagens of these types are used in doses such that about 30 to 70% of the cells are killed.

The mutated or transformed plants then show significantly more rapid growth under low-nitrogen growth conditions than d the conventi nal wild-type plants. Low-nitrogen growth conditions are defined as conditions under which the wild-type shows only very diminished growth. In the case of legumes, an increase in glutamine synthetase, which can be brought about by symbiosis with nodule bacteria and selection, mutation or modification by genetic manipulation, may have particularly beneficial effects in terms of nitrogen utilization.

The plants can be cultivated both in the field and on artificial nutrient media under daylight or with illumination by about 3,000 to 6,000 lux, preferably about 4,000 lux, with a rhythm of 16 hours light and 8 hours dark. This is expediently carried out at temperatures of 15 to 40°C, preferably at 20 to 30°C. In this connection it is immaterial whether the plant-utilizable nitrogen is taken from the air or is obtained from the nutrient medium in the form of nitrite or nitrate.

The stated conditions represent only approximate values, since it is possible to use, according to the invention, crop plants of various species which, of course, may also have growth conditions differing from one another. However, these are known to the expert or can be established in simple preliminary experiments.

The invention is explained in more detail in the examples which follow.

Example 1

The individual steps in the cloning of the glutamine synthetase (GS) gene from Medicago sativa [Tischer et al., loc. cit.] are depicted in Fig. 1. The topmost line of the figure repr sents a section f the plasmid pUC12 GS in which the GS gene is cloned between th BamHI and EcoRI sites of pUC12. For this, use is made of an EcoRI

cleavage site in the 3'-untranslated region which is not shown in the table in Tischer et al., loc. cit.. pUC12 GS is obtained when the 4.2 kb BamHI fragment described therein, and the 1.6 kb BamHI-EcoRI fragment are cloned together into pUC12.

The second line of the figure shows the plasmid pUC12 GS 6 (sectionwise) obtained from pUC12 GS by cutting with BamHI and HindIII and cloning of the 3.85 kb fragment.

The third line of the figure shows a section of the plasmid pUC12 GS 5. This plasmid is obtained from pUC12 GS but cutting with EcoRI, attachment of a SalI linker to the isolated 2.4 kb fragment, and cloning into the SalI cleavage site of pUC12.

The fourth line of the figure is a drawing of a section of the plasmid pUC12 GS 20. This plasmid is obtained by opening pUC12 GS 6 with HindIII, and ligation with the 1.8 kb HindIII fragment from pUC12 GS 5.

The last line of the figure is a section of the plasmid GS-Sal. This is obtained by cutting pUC12 GS 20 with SmaI and BglII, resulting in elimination of the 0.75 kb fragment which codes, inter alia, for the first five amino acids of GS. This is replaced by the following SalI-BglII adapter

			Met	Ser	Leu	Leu	Ser	(Asp)	
TC	GAC	CAA	AAC	ATG	TCT	CTC	CTT	TCA	
	G	GTT	TTG	TAC	AGA	GAG	GAA	AGT	CTA G
(SalI)									(BglII)

which restores the codons for these five amino acids again.

The restriction enzyme SalI is now used to isolate from GS-Sal the GS gene as a single fragment starting with the 6th nucleotide in front of the translation start codon ATG and ending behind a 3'-untranslated region which is about 1200 nucleotides long.

The promoters of the following genes are coupled to the glutamine synthetase gene:

a) Gene 1' of the TR-DNA from Agrobacterium tumefaciens A6 (Velten et al., EMBO J. 3, 2723 (1984))

b) Gene 2' of the TR-DNA from Agrobacterium tumefaciens A6 (Velten et al., EMBO, J. 3, 2723 (1984))

c) ST-LS1, a leaf- or stem-specific gene whose regulation is light-dependent, from Solanum tuberosum (Eckes et al., Mol. Gen. Genet. 205, 15 (1986))

d) 35S transcript from cauliflower mosaic virus (Pietrzak et al., Nucl. Acids Res. 14, 5857 (1986)).

The promoters of the genes mentioned under a and b are cloned into the plasmid pPCV 701 which can be used for the subsequent plant transformation (Koncz et al., Proc. Natl. Acad. Sci., USA 84, 131 (1987)). The ST-LS 1 promoter is cloned as an EcoRI-MboII fragment which is about 1600 bp long (via EcoRI-SmaI) into the polylinker of the intermediate E. coli vector pMPK 110 (Peter Eckes, Dissertation, Cologne University, 1985). The 35 S promoter is likewise cloned into pMPK 110 as an EcoRI-SmaI fragment about 540 bp long (via EcoRI-SmaI).

Behind each of the promoters which are mentioned under a, c and d and have the insertion sequences listed hereinafter there is located a SalI cleavage site, so that the modified GS gene can be inserted into this cleavage site in the correct orientation.

The fusion sites between the promoters and the modified GS gene emerge as follows:

TR1:

Prom ter AAACACCGATATTCATTAATCTTATCTAGTTTCTCAAAAAAA
400 nucleotides

TTCATATCTTCCACACGTGGATCGATCCGTCGAC....
(SalI)

ST-LS1:

Promoter AAGAAGAAAAAAGGTGGGGATCCGTCGAC...
about 1600 nucleotides (SalI)
↑
(MboII/SmaI)

35S:

Promoter GGGTACCCGGGGATCCTCTAGAGTCGAC...
about 500 nucleotides (SmaI) (SalI)

Behind the promoter mentioned under b there is a BamHI restriction cleavage site. After restriction digestion, the resulting protruding DNA ends are filled in with the enzyme DNA polymerase (Klenow fragment), as are the SalI ends of the GS gene. The GS gene is ligated behind the TR2 promoter via the resulting blunt ends of the DNA fragments.

The promoter-GS gene constructs c and d produced in this way are transferred into Agrobacterium tumefaciens with the aid of the intermediate E. coli vector pMPK110 (Peter Eckes, Dissertation, Cologne University, page 91 et seq. (1985)). This so-called conjugation is carried out by the method described by Van Haute et al., EMBO J. 2, 441, (1983). This entails the gen with its regulator signals b ing integrated, by homologous recombination via the sequences of the v ctor pBR322 which are contained in the

pMPK110 vector and in the Ti plasmid pGV3850 Km (Jones t al., EMBO J. 4, 2411 (1985)), into the Ti plasmid. Besid s the gene for resistance to the antibiotic kanamycin, which was already present beforehand and is active in plants, also located on the Ti plasmid pGV3850Km is now the promoter-GS gene construct. Both genes are transferred into tobacco plants using the method called leaf-disc transformation (Horsch et al., Science 227, 1229 (1985)).

The promoter-GS gene constructs a and b on the transformation plasmid pPCV 701 (see above) are mobilized in Agrobacterium tumefaciens pGV3101 by the method described by Konc z et al. (Mol. Gen. Genet. 204, 383 (1986)). The abovementioned leaf-disc method is used to transfer the promoter-GS gene constructs in Agrobacterium, together with, as selectable marker, the kanamycin-resistance gene which is already located on the plasmid pPCV 701, into tobacco plants.

Transformed shoots are selected on the basis of resistance to the antibiotic kanamycin, which is also transferred, and are regenerated to give complete plants. The presence and the expression of the GS gene are demonstrated by DNA analysis (Southern blotting), RNA analysis (Northern blotting) and protein analysis (Western blotting) of the transformed plants.

At the RNA level, the expression of the transferred GS gene from Medicago sativa is about 10 times greater in transformed tobacco plants than in Medicago itself. The GS protein accounts for up to 5% of the total protein in transformed plants. The glutamine synthetase activity is likewise increased, as is evident from the table which follows:

Table

Specific glutamine synthetase activity in tobacco

Type	Activity (ncat/mg total protein)
Tobacco leaf (wild-type)	4
Tobacco leaf (prepared as in Example 1)	20

Example 2

Tobacco plants transformed as in Example 1, and untransformed tobacco plants are cultivated in the (commercially available) culture substrate perlite. The nutrient solution used is the Hoagland medium [Hoagland et al., Proc. Am. Soc. Hort. Sci. 30, 288 (1933)] in which the nitrate content has been reduced to 0.5 mM. Plants overproducing glutamine synthetase show a growth which is about 20%, and thus significantly, more rapid than do untransformed plants.

The same result is obtained on cultivation of the plants on Murashige-Skoog medium (Physiologia Plantarum 15, 473 (1962)) which contains only 0.5 mM KNO_3.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for improving the nitrogen utilizati n in crop plants, which comprises bringing about glutamine synthetase overproduction in the these plants.

2. A method for cultivating crop plants under conditions which are low in plant-utilizable nitrogen, which comprises cultivating crop plants which overproduce glutamine synthetase.

3. A method for generating crop plants, which comprises bringing about glutamine synthetase overproduction in these plants, and providing them with an amount of utilizable nitrogen which does not result in useful growth without glutamine synthetase overproduction.

4. A method as claimed in one or more of claims 1 to 3, wherein the glutamine synthetase overproduction is brought about by selection, mutation or genetic manipulation of the plants or plant cell.

5. The method as claimed in claim 4, wherein the glutamine synthetase overproduction is brought about by insertion of a glutamine synthetase gene/promoter combination into the plant cell.

6. The method as claimed in claim 5, wherein a glutamine synthetase gene obtained from Arabidopsis thaliana, Phaseolus vulgaris, Medicago sativa, Nicotiana plumbaginifolia, Pisum sativum or Nicotiana tabacum is used.

7. The method as claimed in claim 6, wherein the glutamine synthetase gene from Medicago sativa is used.

8. The meth d as claimed in claim 5, wherein a prom ter which has high expression and is btain d fr m plants or from microorganisms which live in symbiosis with plants, or from plant viruses, is used.

9. The method as claim d in claim 8, wherein the promoter to one of the f llowing genes is used:

   a) Gene 1' or gene 2' of the TR-DNA fr m Agrobacterium tumefaciens A6,

   b) ST-LS1 from Solanum tuberosum or

   c) 35S transcript from cauliflower mosaic virus.

10. The use of glutamine synthetase overproduction for improving the utilization of plant-utilizable nitrogen in crop plants.

11. A plasmid containing the glutamine synthetase gene from Medicago sativa and the promoter to one of the following genes:

   a) Gene 1' or gene 2' of the TR-DNA from Agrobacterium tumefaciens A6,

   b) ST-LS1 from Solanum tuberosum or

   c) 35S transcript from cauliflower mosaic virus.

12. A plasmid as claimed in claim 11, which is a derivative of the plasmid pPCV 701 or pMPK 110.

13. Crop plants with improved utilization of plant-utilizable nitrogen, containing a glutamine synthetase structural gene which is controlled by a promoter, with high expression in plants, to one of the following genes

   a) Gene 1' or gene 2' of the TR-DNA from Agrobacterium tumefaciens A6,

   b) ST-LS1 from Solanum tuberosum or

   c) 35S transcript from cauliflower mosaic virus.

14. A crop plant as claimed in claim 13, containing the glutamine synthetase gen from Medicag sativa.

DATED THIS 2nd day of June, 1988

HOECHST AKTIENGESELLSCHAFT

EDWD. WATERS & SONS,
PATENT ATTORNEYS,
50 QUEEN STREET,
MELBOURNE. VIC. 3000.

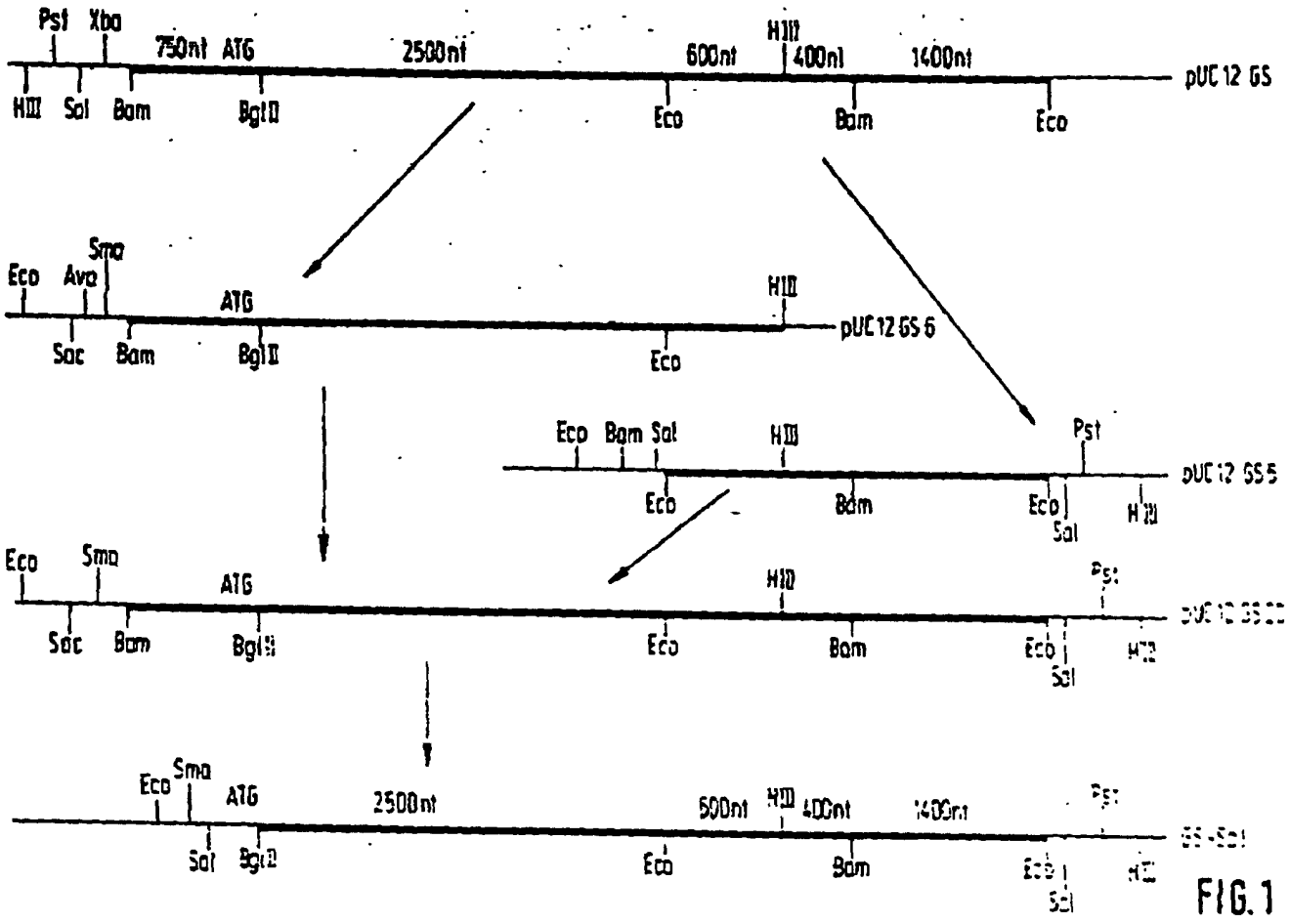
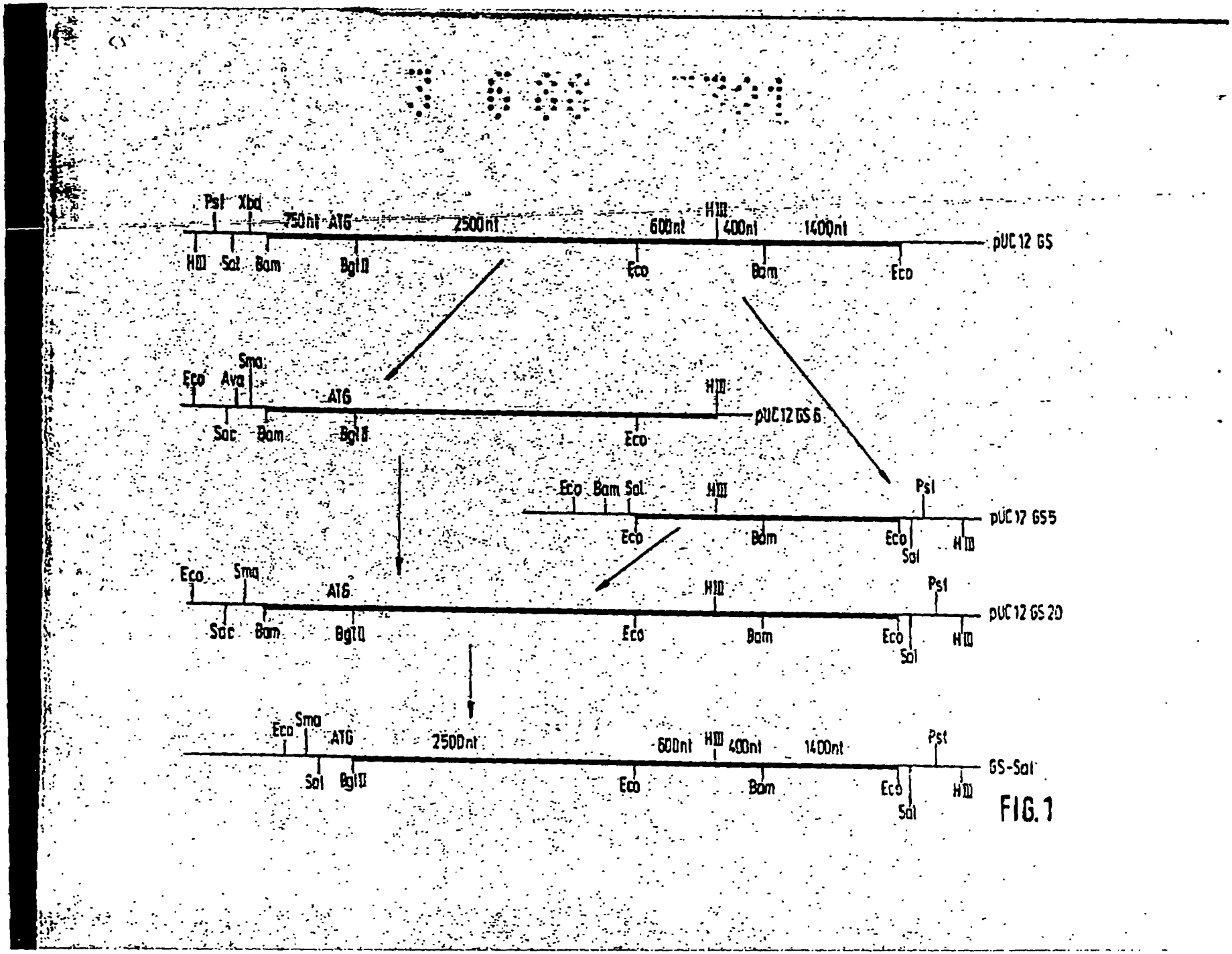

Pst Xba
750nt ATG
2500nt
600nt
HIII
400nt
1400nt
HIII Sal Bam BglII Eco Bam Eco
pUC 12 GS
Eco Ava Sma
ATG
HIII
Sac Bam BglII Eco
pUC12 GS 6
Eco Bam Sal HIII Pst
Eco Bam Eco Sal HIII
pUC 12 GS 5
Eco Sma
ATG
HIII
Pst
Sac Bam BglII Eco Bam Eco Sal
Eco Sma
ATG
2500nt
600nt
HIII
400nt
1400nt
Pst
Sal BglII Eco Bam Eco Sal

FIG. 1


Pst
Xba
750nt
ATG
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pUC 12 GS
HIII
Sal
Bam
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Bam
Eco
Sma
Eco
Ava
ATG
HIII
pUC 12 GS 6
Sac
Bam
BglII
Eco
Eco
Bam
Sal
HIII
Psl
pUC 17 GS 5
Eco
Bam
Eco
Sal
HIII
Eco
Sma
ATG
HIII
Psl
pUC 12 GS 20
Sac
Bam
BglII
Eco
Bam
Eco
Sal
HIII
Eco
Sma
ATG
2500nt
600nt
HIII
400nt
1400nt
Pst
GS-Sal
Sal
BglII
Eco
Bam
Eco
Sal
HIII

FIG. 1